ARMY RESEARCH LABORATORY

Fundamental Interaction Between Au Nanoparticles and Deoxyribonucleic Acid (DNA)

by Molly Karna, Govind Mallick, and Shashi P. Karna

ARL-TR-5219 **June 2010**

NOTICES

Disclaimers

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

Citation of manufacturer's or trade names does not constitute an official endorsement or approval of the use thereof.

Destroy this report when it is no longer needed. Do not return it to the originator.

Army Research Laboratory
Aberdeen Proving Ground MD 21005-5069

ARL-TR-5219 **June 2010**

Fundamental Interaction Between Au Nanoparticles and Deoxyribonucleic Acid (DNA)

Molly Karna
Science and Mathematics Academy at Aberdeen High School

Govind Mallick and Shashi P. Karna
Weapons and Materials Research Directorate, ARL

REPORT DOCUMENTATION PAGE	Form Approved OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED (From - To)
June 2010	Summary	15 June to 9 August 2009

4. TITLE AND SUBTITLE
Fundamental Interaction Between Au Nanoparticless and Deoxytribonucleic Acid (DNA)

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Molly Karna, Govind Mallick, and Shashi P. Karna

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
U.S. Army Research Laboratory
ATTN: RDRL-WMB-D
Aberdeen Proving Ground MD 21005-5069

8. PERFORMING ORGANIZATION REPORT NUMBER
ARL-TR-5219

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Semiconductor quantum dots (QDs) and metal nanoparticles (NPs) have attracted a great deal of attention in the biology community due to their application as fluorescent labels and sensors. The optical properties of NPs allow them to be effective imaging agents for biomolecules. Their biological sensing abilities include identifying target DNA through a linker, followed by color change and electrical signaling. However, size-controlled NPs have the potential to be used as more than just sensors and labels. In order to develop novel applications of NP-biomolecule complex, an understanding of the fundamental interactions between them is critically important. To date, most NP-DNA complexes have been made through linker; i.e., the NPs are attached with DNA via an intermediary molecular link, either functionalized on the surface of NPs or with DNA, or both. The goal of this research is to determine the nature of fundamental interactions that occur directly between NPs and DNA.

15. SUBJECT TERMS
SWNTs, Nanoparticles, AFM, UV-Vis

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE	UU	16	Govind Mallick
Unclassified	Unclassified	Unclassified			19b. TELEPHONE NUMBER (*Include area code*) (410) 306-1901

Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

Contents

List of Figures

1. Introduction and Background

Quantum dots (QDs) and Nanoparticles (NPs) are particles made of metal and/or semiconductor materials that are on the nano size, with diameters ranging from 5–100 nm. The properties of these nanomaterials, which depend on their size and the material they are made from, are usually completely different than the properties of their corresponding bulk materials. There may be anywhere from 1–1000 electrons in a single QD, providing numerous possibilities for their optical and electrical properties.

The unique optical properties of NPs can be beneficial to their biological applications. NPs have broad absorption spectra, ranging from the visible region into the ultraviolet, and narrow, size-tunable photoluminescence spectra. Both of these spectra depend on the size of the NPs. The most attractive optical properties in NPs from a biological aspect appear in those that are approximately 10 nm in diameter or smaller (*1*). When illuminated with ultraviolet (UV) light, such NPs emit extremely bright fluorescent light (*2*). NPs are also effective energy donors in Fluorescent Resonance Energy Transfer (FRET) (*3*), a phenomenon in which energy is transferred between two molecules, from a donor fluorophore to an acceptor chromophore—also known as the quencher—when they come in close proximity to one another. This phenomenon can be used to sense a particular biomolecule from the observation of decrease in fluorescence intensity of NP. Due to such novel optical properties, NPs are used as imaging agents, biolabels, and biosensors exploiting FRET (*3–7*, *10*).

Over the past several years, NPs have been used in various ways to sense deoxyribonucleic acid (DNA) (*1*,*3*,*8*,*9*). This includes the single NP FRET-based DNA nanosensor, which involves target DNA bound to a fluorescent probe, either Cy5 or biotin, which is then bound to another molecule, streptavidin, which binds to the surface of a NP. The targeted DNA is illuminated by FRET, which allows transfer of energy from the NP to the probe, releasing a fluorescent glow (*3*). Fluorescence-based molecular beacons have been developed (*9*) to detect sequence-specific DNA using quantum dots. The beacons, themselves, were DNA-designed to hybridize with the DNA sequence to be detected. NPs were used as a fluorophore, and various quenching moieties were tested to provide the best fluorescence quenching. The molecular beacon DNA was attached to the semiconductor NPs via carboxyl linkage and streptavidin linkage.

Positively charged NP-DNA complexes have also been created as probes themselves, for the detection of nucleic acids (*8*). The complexes were prepared by electrostatic interaction between functionalized NPs and DNA. The NPs were amine-functionalized, creating a cationic nature that allowed the formation of an electrostatic complex with negatively charged DNA (*8*). This biomolecular sensing provides new insight into DNA and linker-functionalized NP interactions. However, the fundamentals of the interactions between NPs themselves—i.e., without any mediating linker—and DNA have yet to be fully understood. Given the vast optical, magnetic,

and electrical properties of NPs, knowledge of their interaction with DNA will create new insight into the world of nanobiotechnology.

In this research, we have investigated the interactions between gold (Au) NPs and single-strand (ss) DNA to develop a fundamental understanding and to identify potential future applications for NP-DNA complexes. These applications include gene therapy, drug delivery to DNA, and an improved method for decoding DNA. A detailed study of NP interactions with DNA is also expected to provide an enhanced understanding of NP interactions with other biomolecules, such as ribonucleic acid (RNA), proteins, and enzymes. NP interactions with these biomolecules are subject to be investigated, once the NP-DNA interactions are determined.

2. Materials and Methods

Colloidal Au NPs were synthesized by reducing hydroaurochloric acid ($HAuCl_4$) by trisodium citrate dihydrate ($Na3C6H5O7.2H_2O$) (*11*) at the University of Mumbai, India, in collaboration with Professor Aswini Srivastava's group. The suspended NPs were sonicated for 15 min to obtain a uniform distribution and cluster free nanoparticles. Fairly uniform sized Au NPs capped by citrate ions were obtained. About 25 μl of the suspension was spin-coated at a rate of 120 rpm for 6 min onto a silicon substrate and dried in a dessicator under clean environment. Figure 1 shows the scanning electron microscopic (SEM) image of Au NPs with an average size of ~20–25 nm.

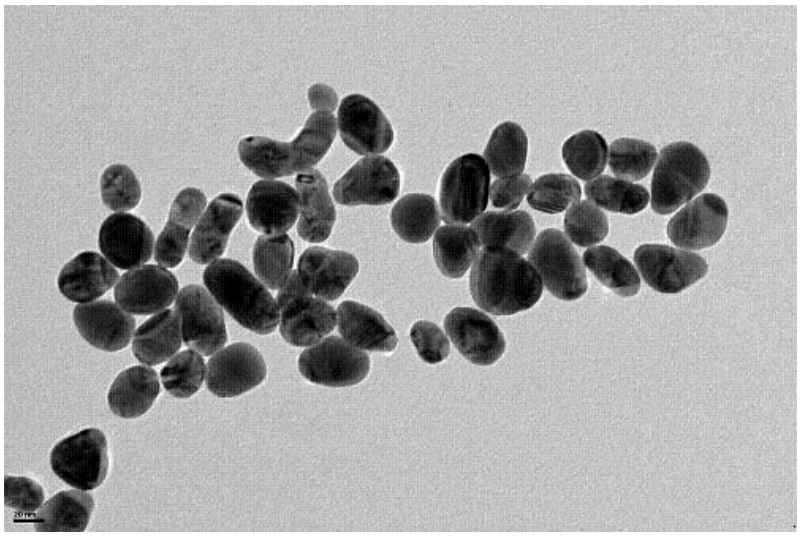

Figure 1. Gold nanoparticles viewed under a scanning electron microscope.

1 mM DNA (Ba813 Target 30, 30 bp; Integrated DNA technologies) solution was prepared using 5 ml of phosphate buffer (0.1 M, pH 6.35). One drop (~25 μl) of the solution was then spin-coated onto an Au substrate at a rate of 120 rpm for 3 min. The surface characterization of the

Au substrate with DNA was performed using tapping mode atomic force microscopy (AFM). The ss DNA with an average height of ~2 nm is shown in figure 2.

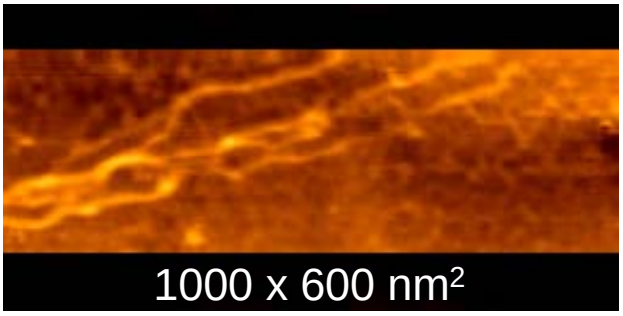

Figure 2. Tapping mode AFM image of ssDNA on Au subtrate.

The Au NP and the DNA solutions were mixed at a 1:1 ratio and spin-coated on a freshly cleaved mica substrate, as described earlier. The dried substrate containing the NPs and DNA was imaged using tapping mode AFM. A series of images (figure 3) were obtained that showed prominent interaction between Au NPs and DNA. The average heights of Au NPs and DNA were ~4 nm and 2 nm, respectively.

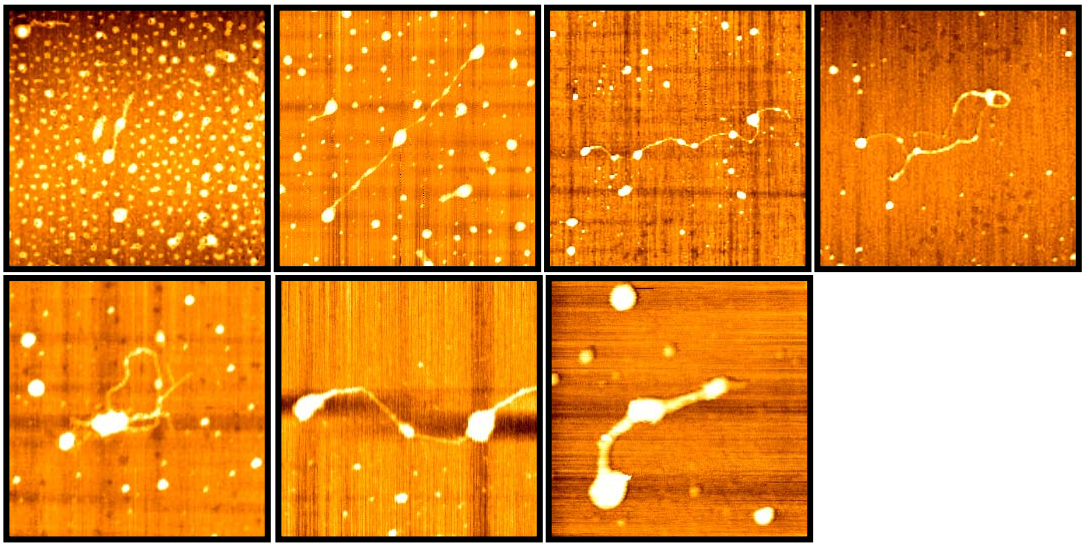

Figure 3. Various tapping mode AFM images of Au NPs-DNA hybrid system on mica surface (clockwise from top left: 5x5 μm^2, 5x5 μm^2, 3.5x3.5 μm^2, 3x3 μm^2, 2.5x2.5 μm^2, 1x1 μm^2, and 1x1 μm^2 scan size).

The spin-coated substrates for each sample were probed under an Autoprobe Atomic Force Microscope (AFM; Veeco, CP-II). The emission spectra of each sample at 250 nm excitation energy were obtained using JY Horiba Fluoromax-3 Spectrofluorometer.

3. Results and Discussion

3.1 Spectral Analysis

The Au NPs showed maximum emission at a wavelength of about 461 nm (figure 4a). Similarly, the maximum emission of DNA was at the wavelength of 381 nm (figure 4b), which was comparatively at much lower intensity (~27000 counts per second) than Au NP (~250000 cps). The emission spectrum of the mixture of NP and DNA showed (figure 4c) very broad peak at low intensity (~70000 cps). When the graph was exposed between 330 and 530 nm, two distinct peaks of DNA (~396 nm at ~50000 cps) and Au NP (~469 nm at ~70000 cps), respectively, could be clearly distinguished (figure 4c (inset)). The broad and overlapping emission spectra of Au NP and DNA reveal that Au NPs at lower intensity have strong affinity to DNA. The possible reason of positive shift in the wavelengths of both Au NP and DNA for Au NP-DNA mixture is unknown and requires further investigation. However, the broad and overlapping emission spectra of Au NP and DNA reveal the strong interaction between the Au and DNA.

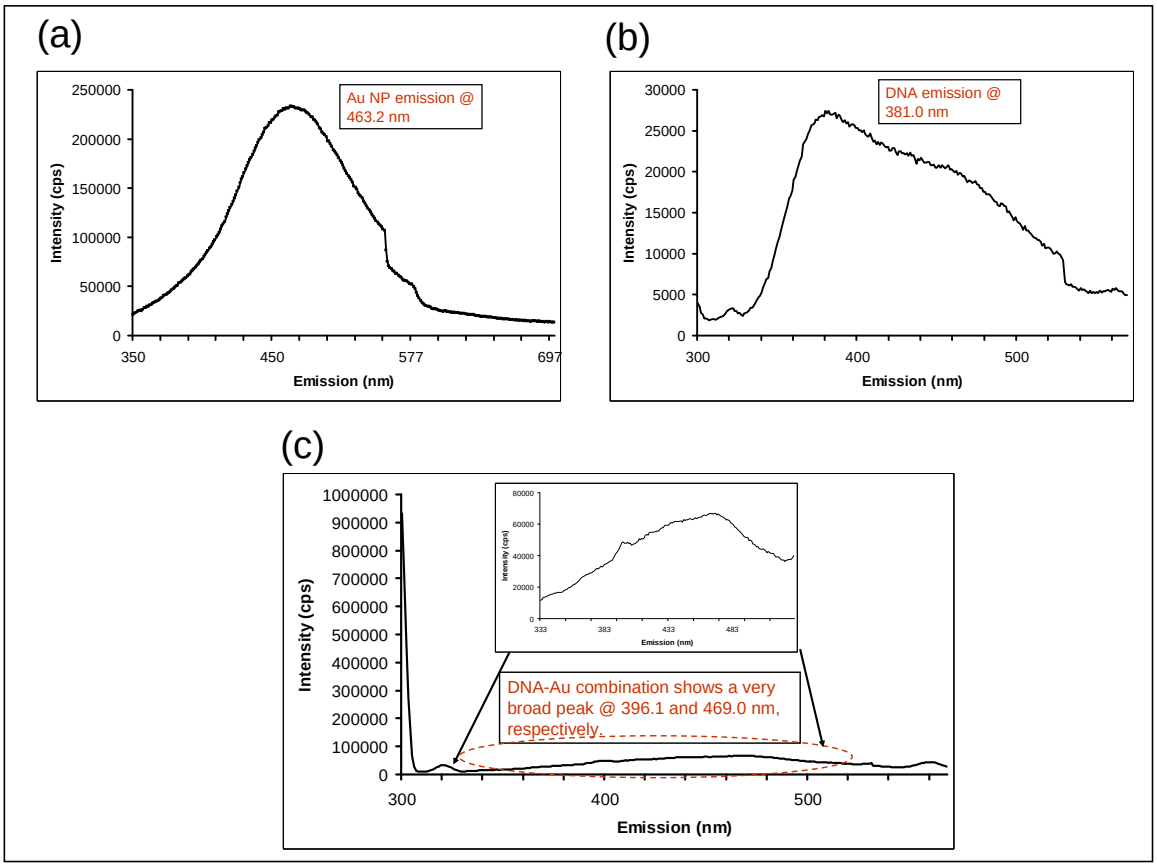

Figure 4. Fluorescence emission spectra of (a) Au NP, (b) ssDNA, and (c) Au NP-DNA. The emission spectra at ~460 nm and ~380 nm for Au NP and DNA, respectively reappear in the Au NP-DNA spectrum as a broader peak, shown in the inset of (c).

3.2 Microscopic Analysis

The morphology and surface properties of the Au NPs (figure 1), DNA (figure 2), and the Au NP-DNA (figure 3) complex on different substrates reveal the uniform heights; the diameter, however, varied considerably. The heights of the NPs ranged between 2–6 nm with average height to be 4 nm (not shown here). The diameter ranged between 30 to 250 nm, with an average of 100 nm. This observation suggested the clustering/agglomeration of small NPs and nanorods into larger particles. Similarly, the height and the diameter of the ssDNA ranged between 1–3 nm and 25–75 nm, respectively, suggesting that there were possibility of more than one single stranded DNA lined parallel to each other. It is also worth noting that AFM tend to exaggerate the measurement in x or y direction due to the x-y movement of the scanner.

The Au NP-DNA sample showed both Au NPs and DNA oligomer strands. AFM images (figure 3) show that the DNA and Au NPs do, indeed, interact with one another. The DNA appears to link to larger Au NPs of an overall average size 150 nm. Smaller NPs appear more frequently in the images than Au NP-DNA linked complexes.

4. Summary and Conclusions

The results indicate that the citrate-capped Au NPs naturally link with ssDNA. The exact nature of the interaction needs to be determined, though it can be speculated that the attractive forces between the Au NPs and ssDNA are a type of intermolecular attractive force rather than a type of chemical bond. Further characterization of the Au NP-DNA complex needs to be performed to substantiate the previous results and determine the type of the interaction.

5. References

1. Algar, W. R.; Krull, U. J. *Anal. Bioanal. Chem.* **2008**, *391*, 1609–1618.
2. Zhang, C. Y.; et al. *Nat. Mat.* 4 **2005**, *8*, 26–831.
3. Tran, P. T.; et al. *Phys. Stat. Sol.* **2002**, *229*, 427–432.
4. Medintz, I. L.; et al. *Nat. Mat.* 2 **2003**, 630–638.
5. Medintz, I. L.; et al. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 9612–9617.
6. Deniz, A. A.; et al. *Proc. Natl. Acad. Sci. USA* **1999**, *96***,** 3670–3675.
7. Wang, Y.; et al. *Mat. Today* 8 **2005**, 20–31.
8. Lee, J.; et al. *ChemPhysChem.* 10, **2009**, 806–811.
9. Cady, N. C.; et al. *Mol. Cell. Probes* **2007**, *21*, 116–124.
10. Bauer, G.; et al. *Inst. of Phys. Publ.* **2003**, *14*, 1229–1311.
11. Mcfarland, A. D.; et al. *J. Chem. Educ.* **2004**, *81*, 544A.

List of Symbols, Abbreviations, and Acronyms

AFM	atomic force microscopy
Au	gold
DNA	deoxyribonucleic acid
FRET	Fluorescent Resonance Energy Transfer
HAuCly	hydraurochloric acid
NPs	nanoparticles
QDs	Quantum dots
RNA	ribonucleic acid
SEM	scanning electron microscopic
ss	single strand
UV	ultraviolet

NO. OF COPIES	ORGANIZATION
1 ELECT	ADMNSTR DEFNS TECHL INFO CTR ATTN DTIC OCP 8725 JOHN J KINGMAN RD STE 0944 FT BELVOIR VA 22060-6218
1	DEPUTY DIRECTO FOR S&T DEFENSE THREAT REDUCTION AGENCY CHEM/BIO TECH ATTN S R BOSCO 8725 JOHN J. KINGMAN RD STOP 6201 FT BELVOIR VA 22060-5201
1	ARL HRED ATTN RDRL HR P H DIETZ BLDG 459 ABERDEEN PROVING GROUND MD 21005
1	DEFENSE THREAT REDUCTION AGENCY ATTN STOP 6201 C BASS 8725 JOHN J KINGMAN RD FT BELVOIR VA 22060-6201
1	EDGEWOOD CHEMICAL BIOLOGICAL CTR ATTN AMSRD ECB RT J VALDES 5183 BLACKHAWK RD ABERDEEN PROVING GROUND MD 21010-5424
1	EDGEWOOD CHEMICAL BIOLOGICAL CTR SENIOR RSRCH SCIENTIST (ST) – CHEMISTRY ATTN AMSRD ECB RT D A FOUNTAIN 5183 BLACKHAWK RD ABERDEEN PROVING GROUND MD 21010-5424
1	NIGHT VISION & ELECTRONIC SENSORS DIRECTORATE ATTN AMSRD CER NV OPS TPP J URIBE 10221 BURBECK RD FT BELVOIR MD 22060-5806
1	US ARMY AVIATION & MISSILE COMMAND ATTN AMSRD AMR SG P RUFFIN BLDG 5400 REDSTONE ARSENAL AL 35898
1	US ARMY CERDEC/NVESD ATTN AMSRD CER NV 10221 BURBECK RD FT BELVOIR VA 22060
1	US ARMY ECBC ATTN AMSRD ECB RT 5183 BLACKHAWK RD BLDG E-5951 ABERDEEN PROVING GROUND MD 21010-5424
1	US ARMY ENGINEER RSRCH AND DEV CTR ENVIRON LAB ATTN J A STEEVENS 3909 HALLS FERRY RD VICKSBURG MS 39180
1	US ARMY ERDC CONSTRUCTION ENGRG RSRCH LAB ATTN A KUMAR PO BOX 9005 CHAMPAIGN IL 61826-9005
1	US ARMY INFO SYS ENGRG CMND ATTN AMSEL IE TD A RIVERA FT HUACHUCA AZ 85613-5300
1	US ARMY MEDICAL RSRCH INSTITUTE OF INFECTIOUS DISEASE ATTN F LEBEDA 1425 PORTER AVE FT DETRICK MD 21702-5011
1	US ARMY NATICK SOLDIER CENTER ATTN AMSRD NSC KANSAS STREET NATICK MA 01760

NO. OF COPIES	ORGANIZATION
1	US ARMY NATICK SOLDIER CENTER ATTN AMSRD NSC SS L SAMUELSON KANSAS STREET BLDG 78 NATICK MA 01760-5056
2	US ARMY NATICK SOLDIER CTR ATTN AMSRD NSC SS MS B DECRISTOFANO ATTN AMSRD NSC SS MS S FOSSEY 15 KANSAS ST NATICK MA 01760
2	US ARMY NATICK SOLDIER CTR ATTN AMSRD NSC SS MS B KIMBALL ATTN AMSRD NSC SS MS M ROYLANCE NATICK MA 01760-5019
1	US ARMY RDECOM ATTN AMSRD CER CS A BALLATO CHIEF SCIENTIST FT MONMOUTH NJ 07703-5201
1	US ARMY RDECOM CERDEC NVESD ATTN AMSRD CER NV OD J RATCHES 10221 BURBECK RD STE 430 FT BELVOIR VA 22060-5806
2	US ARMY RDECOM/AMRDEC ATTN AMSRD AMR SG NC ATTN AMSRD AMR SG NC J C HOLT REDSTONE ARSENAL AL 35898-5254
1	US ARMY RSRCH LAB ATTN AMSRD CER ST AS FM J BOKSINER FT MONMOUTH NJ 07703-5201
1	US ARMY RSRCH LAB CERDEC STCD ATTN D MORRIS FT MONMOUTH NJ 07703-5201

NO. OF COPIES	ORGANIZATION
1	US MILITARY ACADEMY DEPT OF CHEMISTRY AND LIFE SCIENCES ATTN LTC J INGRAM BLDG 753 OFFICE 24B WEST POINT NY 10996
1	AFB RSRCH LAB (711 HPW/RHPB) SCI, APPLD BIOTECH APPLD ATTN S HUSSAIN 2729 R ST AREA B GLDG 837 WRIGHT PATTERSON AFB OH 45433-5707
30	US ARMY RSRCH LAB ATTN RDRL WMB D G MALLICK (15 COPIES) ATTN RDRL WM S KARNA (15 COPIES) BLDG 4600 ABERDEEN PROVING GROUND MD 21005
1	US ARMY RSRCH LAB ATTN RDRL CIM G T LANDFRIED BLDG 4600 ABERDEEN PROVING GROUND MD 21005-5066
9	US ARMY RSRCH LAB ATTN IMNE ALC HRR MAIL & RECORDS MGMT ATTN RDRL CIM L TECHL LIB ATTN RDRL CIM P TECHL PUB ATTN RDRL SEE I K K CHOI ATTN RDRL SEE M P SHEN ATTN RDRL SER J MAIT ATTN RDRL SER L M DUBEY ATTN RDRL SER L S KILPATRICK ATTN RDRL SER P AMIRTHARAJ ADELPHI MD 20783-1197
TOTAL:	68 (67 HCS, 1 ELECT)

INTENTIONALLY LEFT BLANK.